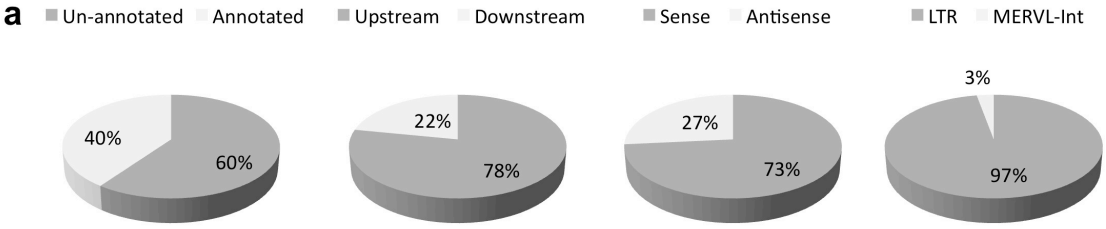
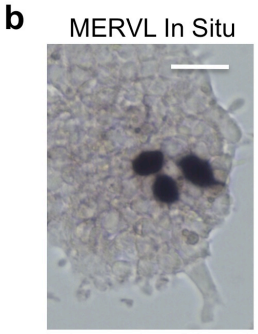
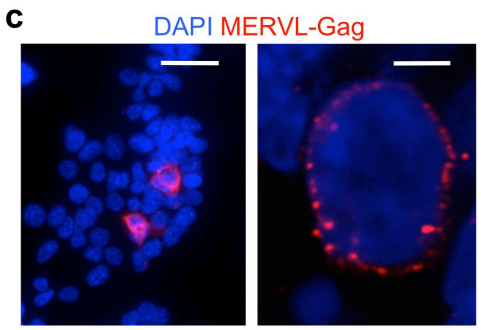
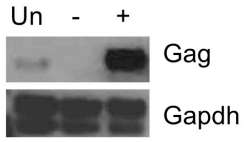
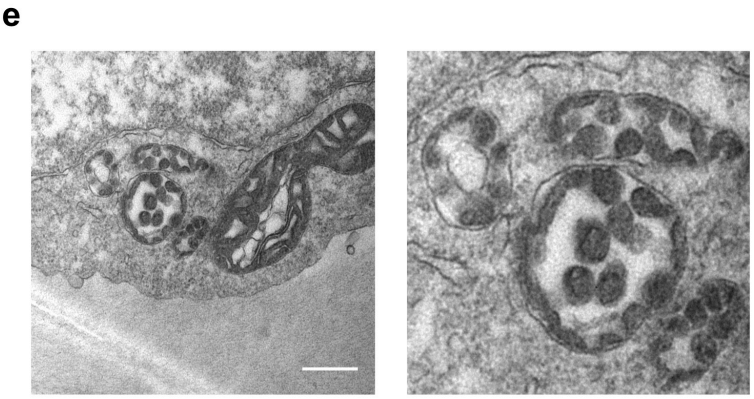
Supplementary Figure 1

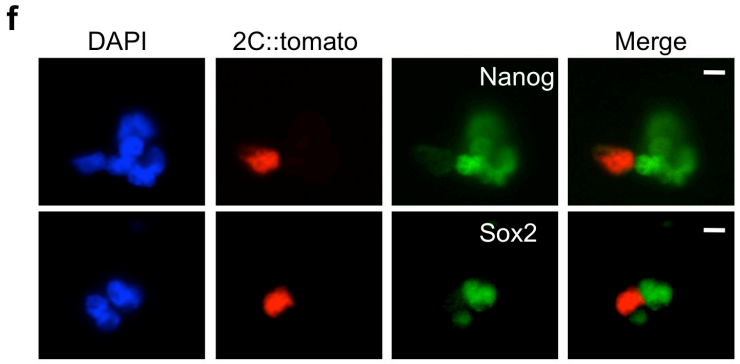






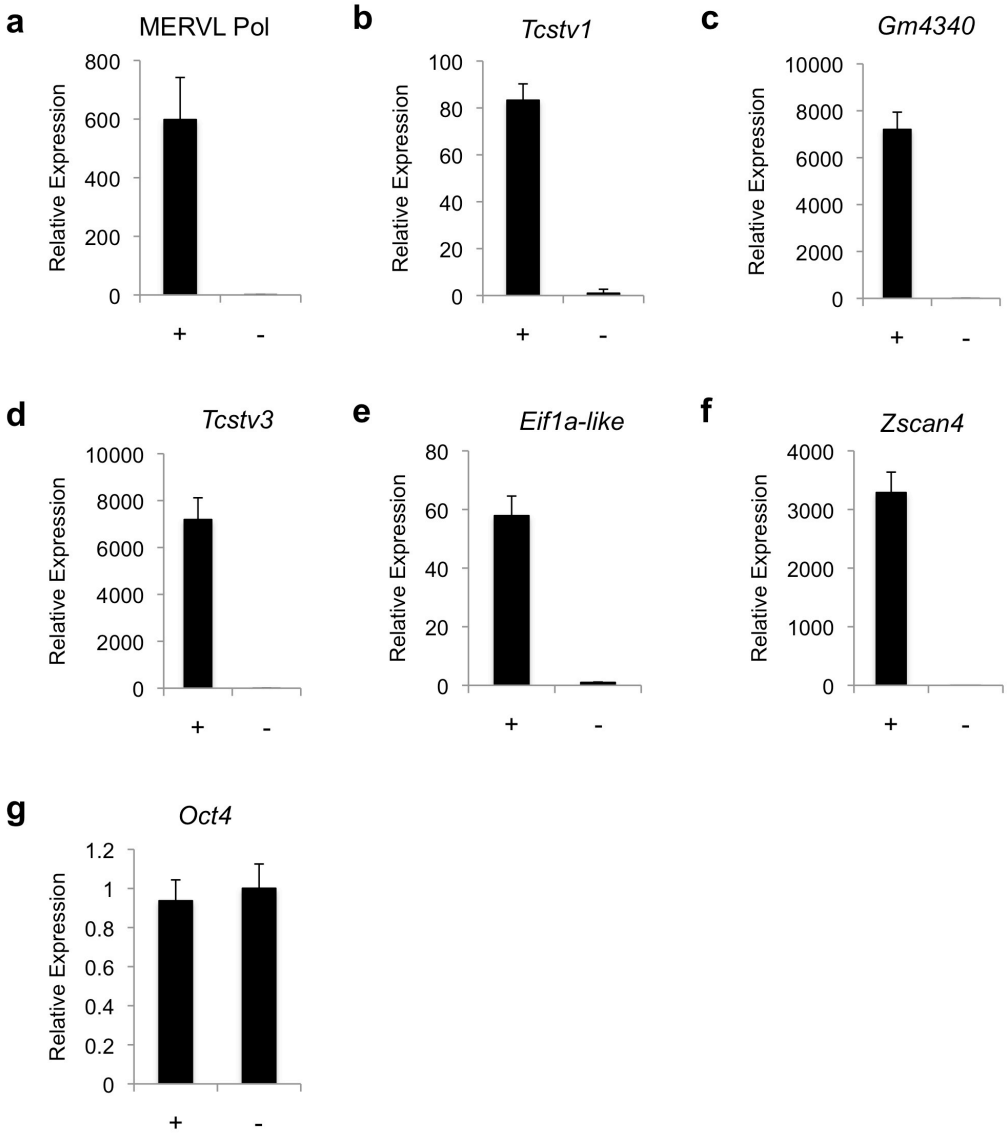






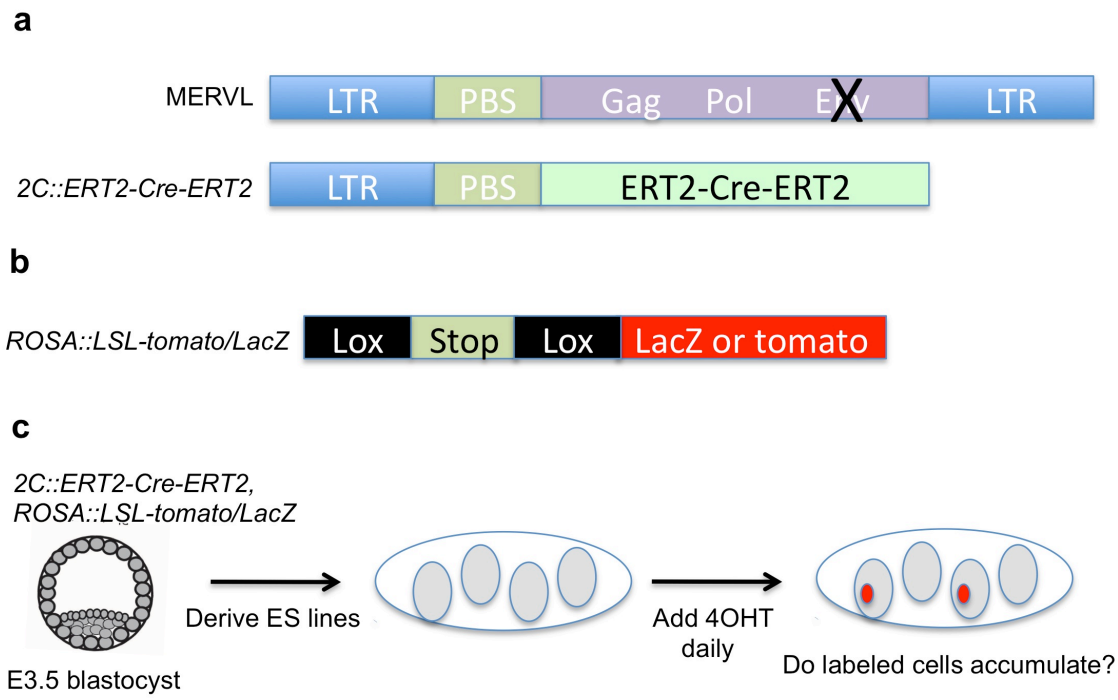
Supplementary Figure 1. a, MERVL-linked chimeric transcripts were grouped according to whether the transcripts were previously annotated, whether the MERVL portion of the transcript was the upstream or downstream portion of the chimera, whether the LTR was sense or antisense to the gene, and whether the MERVL portion of the chimera was derived from the LTR or part of the viral internal sequence (-int). **b-c,** MERVL expression was visualized in wild type mouse ES cells by **(b)** in-situ hybridization (Scale bar 10μm**)** and **(c)** immunofluorescence microscopy with antibodies recognizing MERVL-Gag protein (ES colony left; Scale bar 10μm, single ES cell, right, Scale bar 2μm). **d,** Unsorted (Un) *2C::tomato* ES cultures or *2C::tomato* $^{+}$ and $^{-}$ cells collected by FACS were subject to immunoblots with indicated antibodies to demonstrate accuracy of the reporter. **e,** Transmission electron microscope image of a *2C::tomato*$^{+}$ ES cell collected by FACS. Epsilon particles encoded by MERVL fill the lumen of the endoplasmic reticulum. Scale bar 500nm. **f,** *2C::tomato* ES cells were immunostained with Sox2 and Nanog antibodies and counterstained with DAPI, demonstrating that *2C::tomato*$^{+}$ cells express low levels of pluripotency markers. Scale bar 10μm.

Supplementary Figure 2



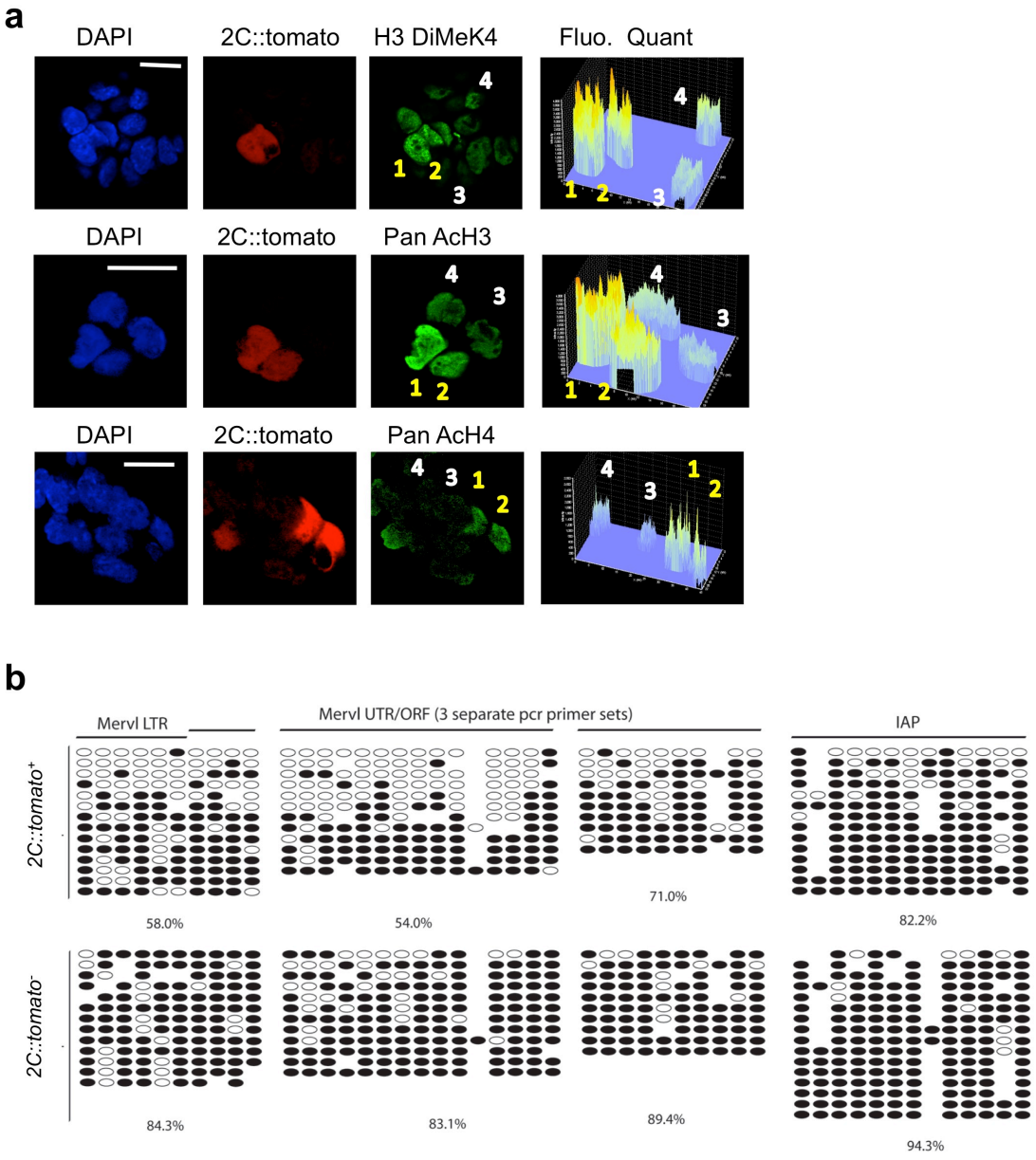
Supplementary Figure 2: a-g The relative expression of the indicated genes/retroelements (compared with *Gapdh*) was determined using QRT-PCR on *2C::tomato*$^{+}$ and $^{-}$ cells collected by FACS. Error bars represent s.d., n=3.

Supplementary Figure 3



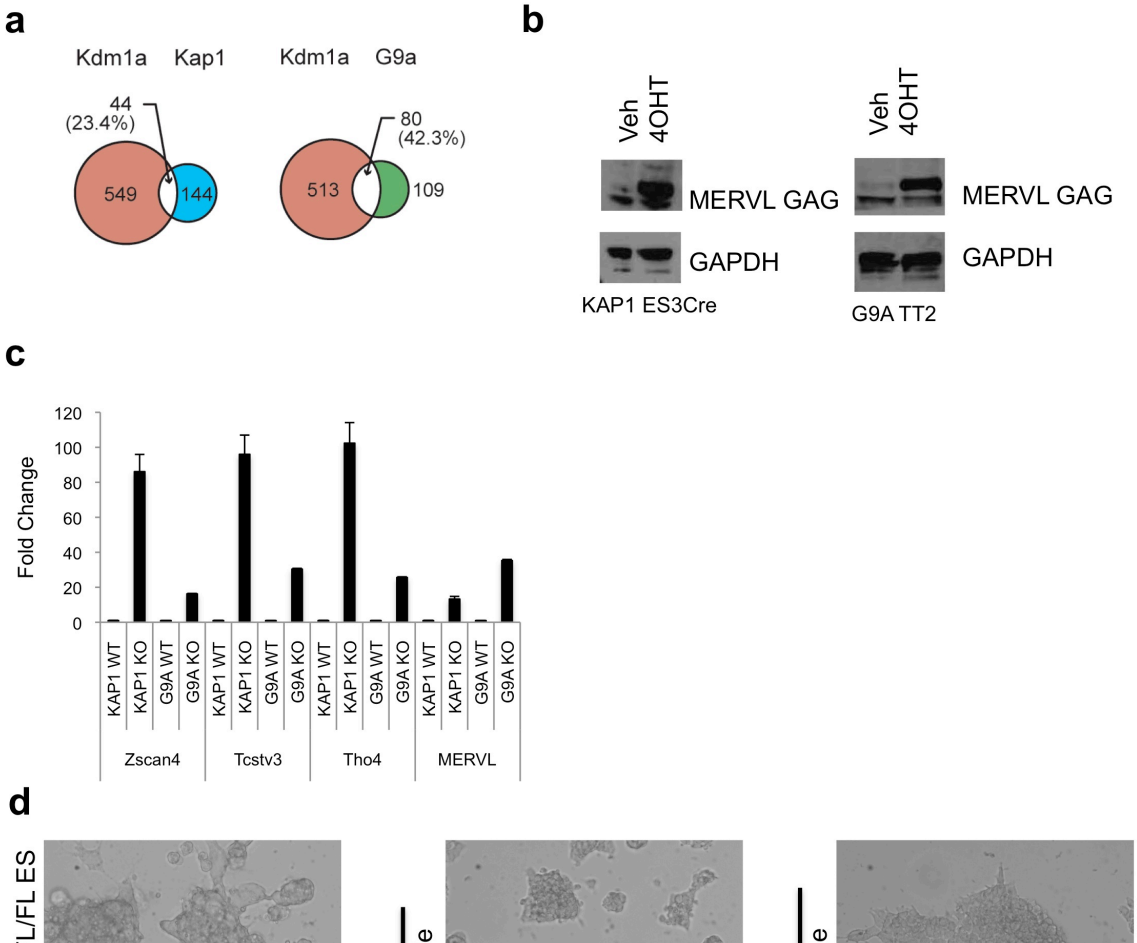
Supplementary Figure 3: **a,** Schematic of a typical MERVL retro-element and the *2C::ERT2CreERT2* transgene. **b,** Schematic of ROSA::LSL-LacZ/tomato transgene. **c,** Schematic of fate mapping strategy to observe activation of 2C genes within a population of ES cells. **d** *2C::ERT2CreERT2* ES cells were treated with 4OHT for 2 hours and immunostained with MERVL-Gag and Cre recombinase antibodies as indicated and counterstained with DAPI. Scale bar 25µm.

Supplementary Figure 4



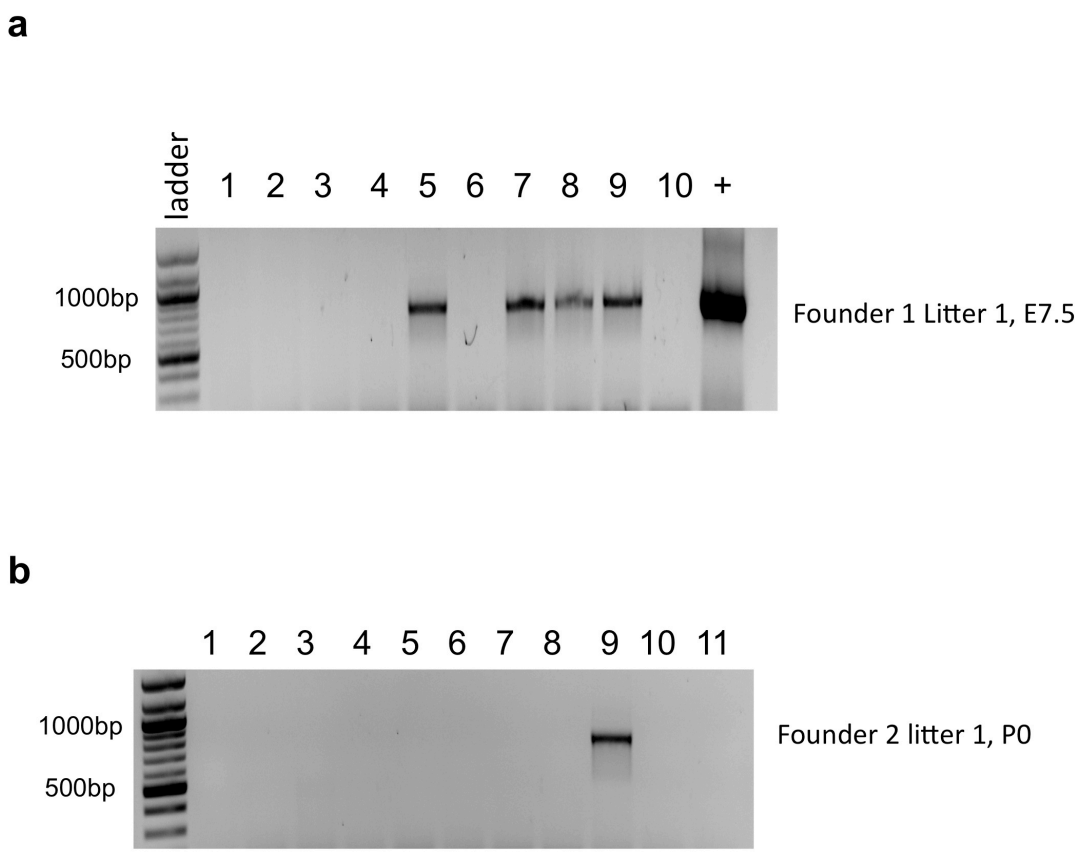
Supplementary Figure 4: a, *2C::tomato* ES cultures were immunostained with histone modification-specific antibodies and fluorescent secondary antibodies as indicated. The fluorescence intensity was quantified for two tomato^{+} cells (1, 2 yellow) or 2 tomato^{-} cells (3, 4 white) in each image using Fluoview. Scale bar 20μm. **b,** *2C::tomato* $^{+}$ and $^{-}$ cells were collected by FACS and subjected to bisulfite sequencing analysis to determine the methylation status of MERVL and IAP retroviruses. The percentage of methylated cytosines for each primer set is shown.

Supplementary Figure 5



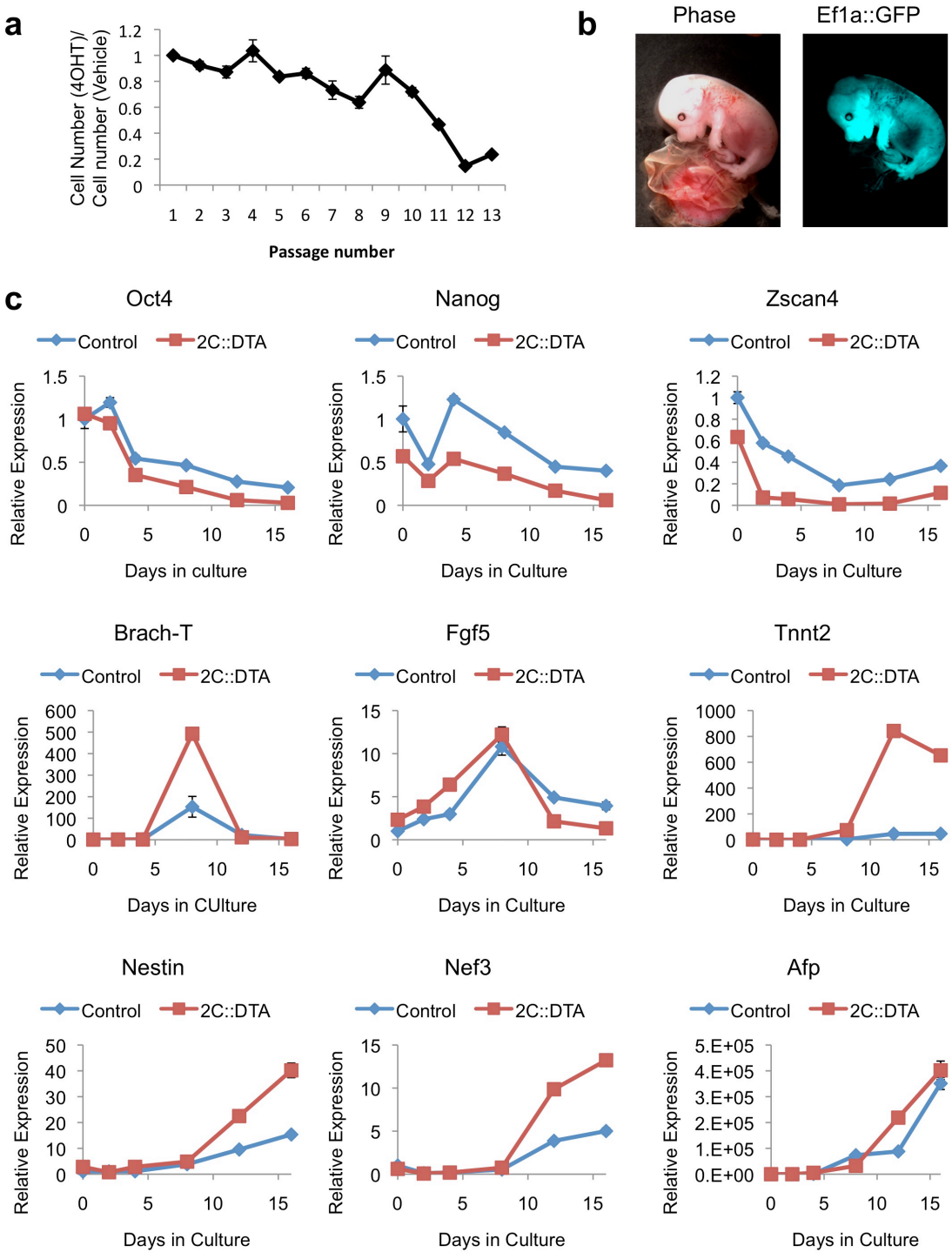
Supplementary Figure 5: a, Pair-wise comparison of re-activated genes in *Kdm1a, Kap1, and G9a* mutant ES cells. **b,** *Kap1* ES3Cre ES cells or *G9a* TT2 ES cells were treated with vehicle or 4OHT to delete *Kap1* and *G9a*, respectively, and cell extracts were subject to immunoblot with the indicated antibodies. **c.** The fold change in expression of the indicated 2C genes in *Kap1* and *G9a* KO ES cells was determined by QRT-PCR. Error bars represent s.e.m., n=3 **d,** In situ hybridization using a MERVL probe was performed on *Kdm1a, KAP1*, and *G9A* mutant ES cell lines and corresponding wild type ES lines.

Supplementary Figure 6



Supplementary Figure 6: a-b, ES cells derived from *2C::tomato* transgenic mice were subject to FACS to collect tomato^{+} cells, and these cells were immediately injected into wild type blastocysts to generate chimeric mice. Chimeric founder mice were then mated with wild type mice and E7.5 (a) or P0 (b) litters (from two separate founders) were subject to genotyping PCR with a tomato primer set. These data indicate that *2C::tomato*$^{+}$ cells contribute to the germ lineage.

Supplementary Figure 7



Supplementary Figure 7: a, 2C::ERT2-Cre-ERT2, ROSA LSL::DTA ES cells were cultured in the presence or absence of 4OHT, and the relative cell numbers were plotted at each passage. Error bars represent s.e.m., n=3. **b,** *2C::ERT2CreERT2*, *LSL::DTA* ES cells were treated with 4OHT for 20 passages prior to injection into blastocyst stage embryos which were implanted into pseudopregnant females. Prior to injection (at passage 18), cells were infected with a lentivirus expressing an Ef1a::GFP reporter. Chimeric embryos were harvested at E13.5 and visualized using fluorescence microscopy. **c,** *2C::ERT2CreERT2*, *LSL::DTA* ES cells treated with vehicle (control) or 4OHT (2C::DTA) for 20 passages were grown in suspension in the absence of Lif to induce differentiation. Samples were harvested at indicated time points for triplicate QRTPCR analysis using the indicated primers and *Gapdh* to normalize. Expression was plotted relative to control sample at the day 0 time point. Error bars represent s.d., n=3.

Supplementary Movie Legends

Supplementary Movie 1: The *2C::tomato* reporter is restricted to the zygote and 2C/4C stage. The *2C::tomato* reporter was injected into fertilized eggs, which were then developed *in vitro* for 24 hours before imaging overnight.

Supplementary Movies 2: *2C::tomato* is transiently expressed in ES cultures. *2C::tomato* (-) cells were collected by FACS and plated. After 2 hours, live imaging was performed overnight to visualize the appearance of *2C::tomato* (+) cells.

Supplementary Movies 3: Entrance into the *2C::tomato* (+) state is more rapid in *Kdm1a* mutant ES cells. *2C::tomato* (-) cells were collected by FACS in cells depleted with *Kdm1a* (using Cre-mediated excision) and plated. After 2 hours, live imaging was performed overnight to visualize the appearance of *2C::tomato* (+) cells.